Supplemental Information

Human neuroglobin and H64A distal variant: how mutation and pH affect the heme pocket.

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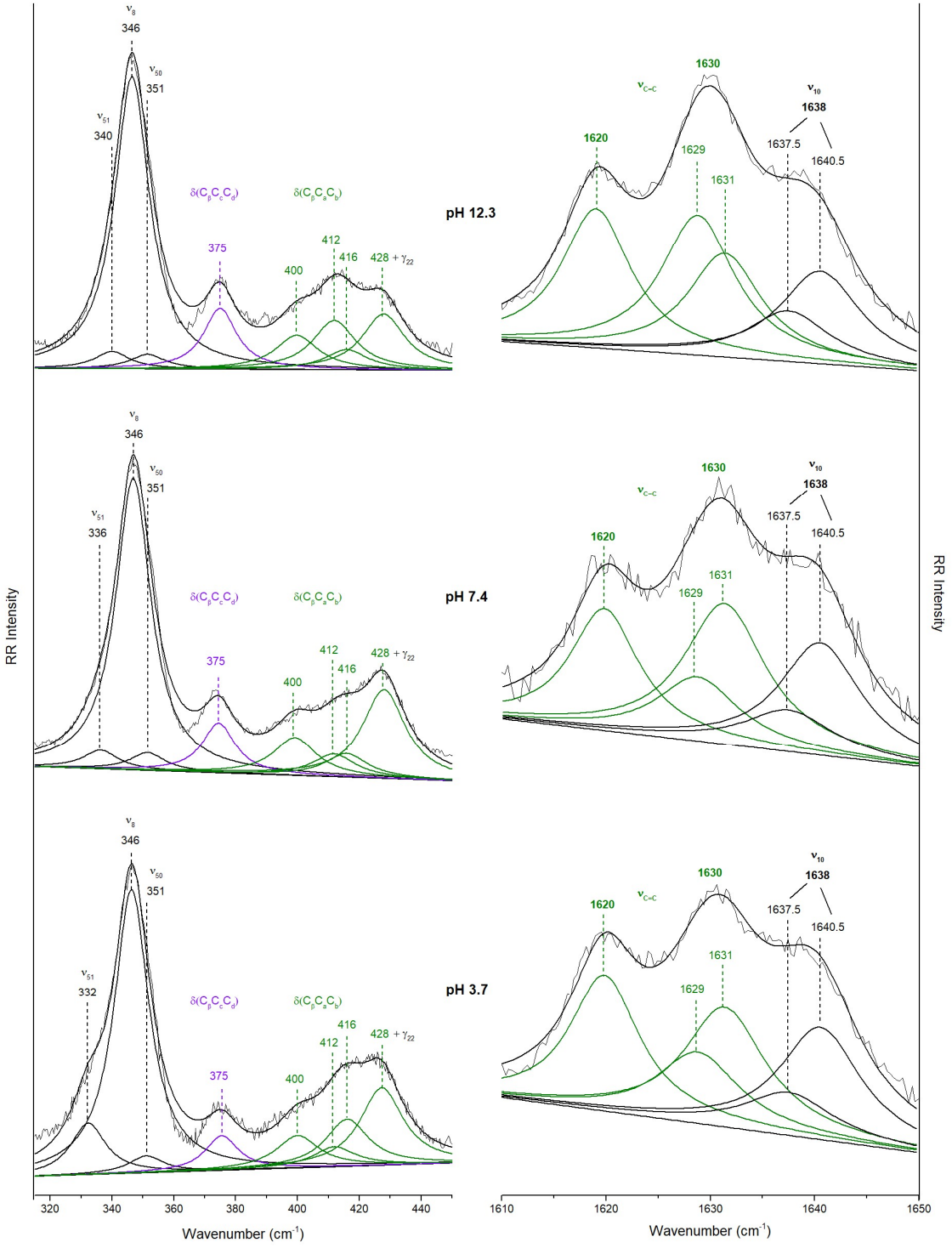

Figure S1. Spectral deconvolution of RR spectra of the ferric hNgb-WT obtained at different pH values in the low (left) and high wavenumber regions (right). The propionate bending modes are reported in purple, while the vinyl bending and stretching vibrations are reported in green. The bands are assigned according to Refs. [1-3]. It is worth noticing that the band at 428 cm^{-1} could arise from the overlap of the vinyl bending vibration and the γ_{22} mode. The corresponding bandwidths are about 13 and 17 cm^{-1} for the

propionate and vinyl bending modes, respectively, and 12 cm^{-1} for the vinyl stretching modes, while are within the 11-14 cm^{-1} range for the other bands.

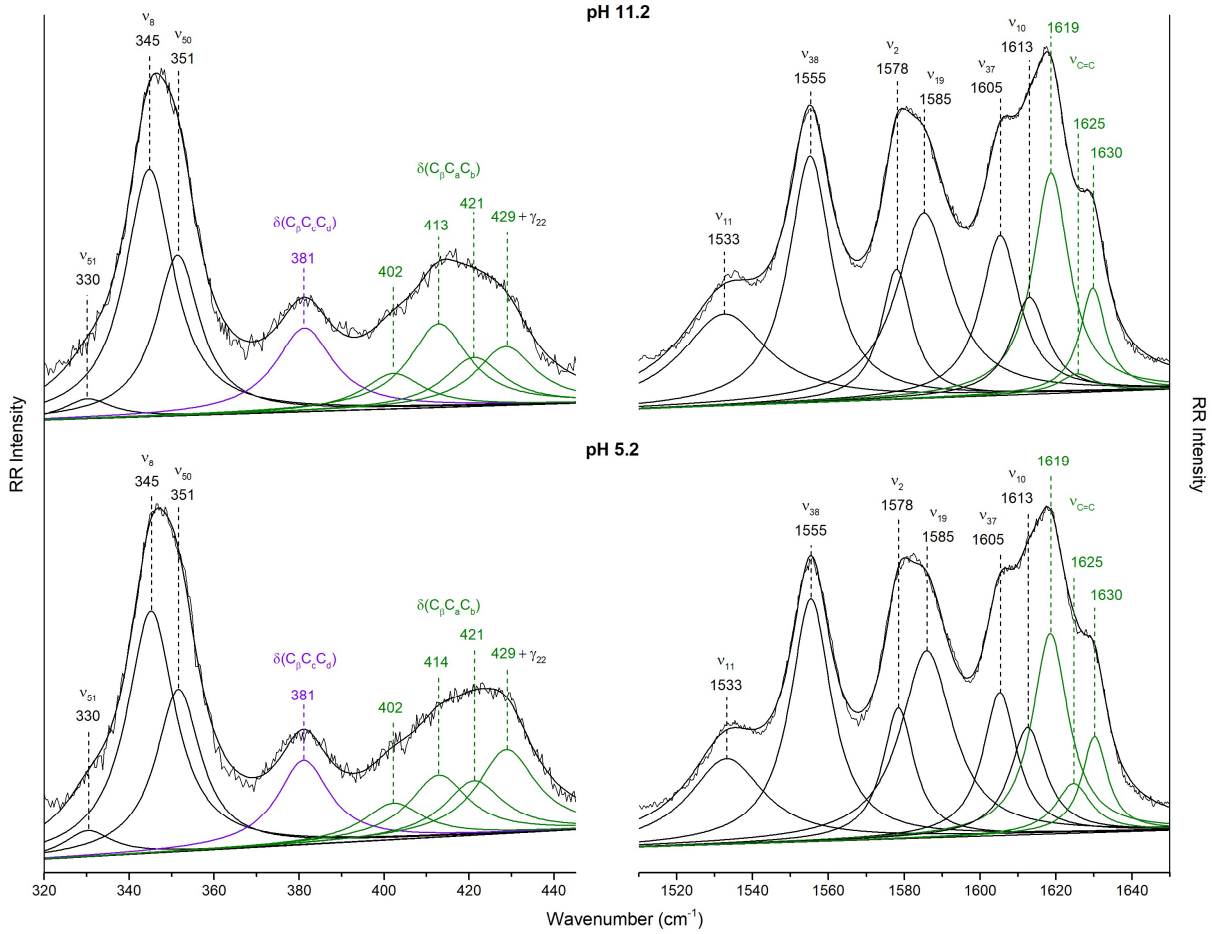

Figure S2. Spectral deconvolution of RR spectra of the ferrous hNgb-WT obtained at different pH values in the low (left) and high wavenumber regions (right). The propionate bending modes are reported in purple, while the vinyl bending and stretching vibrations are reported in green. The bands are assigned according to Refs [1-3]. It is worth noticing that the band at 429 cm^{-1} could arise from the overlap of the vinyl bending vibration and the γ_{22} mode. The corresponding bandwidths are about 17 cm^{-1} for the propionate and vinyl bending modes and 12 cm^{-1} for the vinyl stretching modes, while are within the 10-14 cm^{-1} range for the other bands. The only exception is represented by the ν_{11} mode at 1533 cm^{-1} whose bandwidth is 28 cm^{-1}.

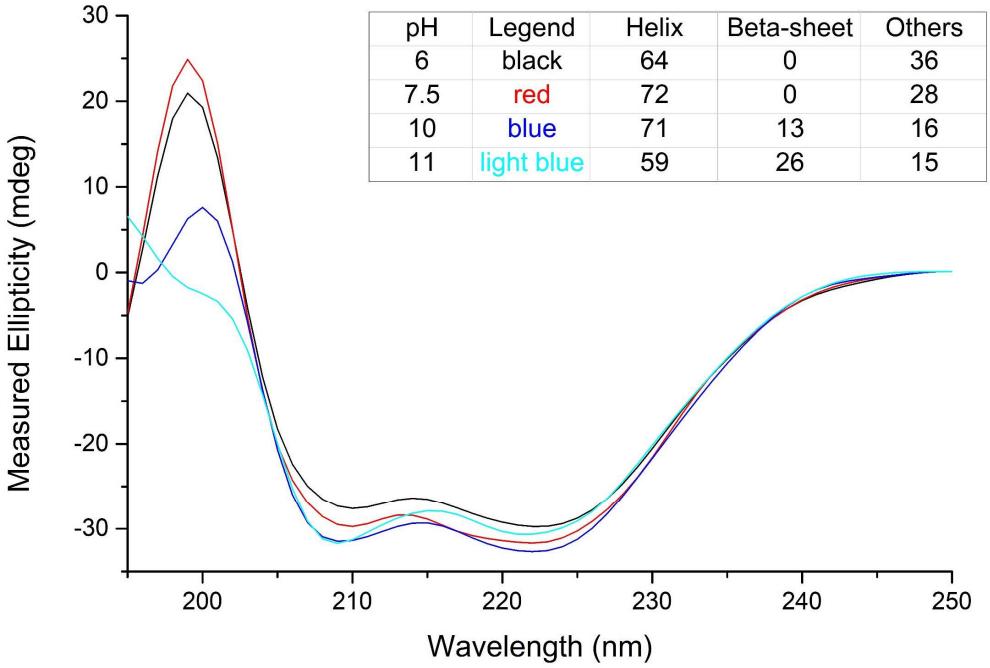

Figure S3. Far UV CD spectra of H64A variant at between pH 6 and 11. The far-UV CD spectra of H64A variant between pH 6 and 11 feature the negative bands at 208 and 222 nm typical of α-helical structures. The amount of the latter are almost unchanged up to pH 10 and slightly decrease at pH 11. The percentages of secondary structure elements at different pH values reported in the table were determined using the BeStSel software [4].

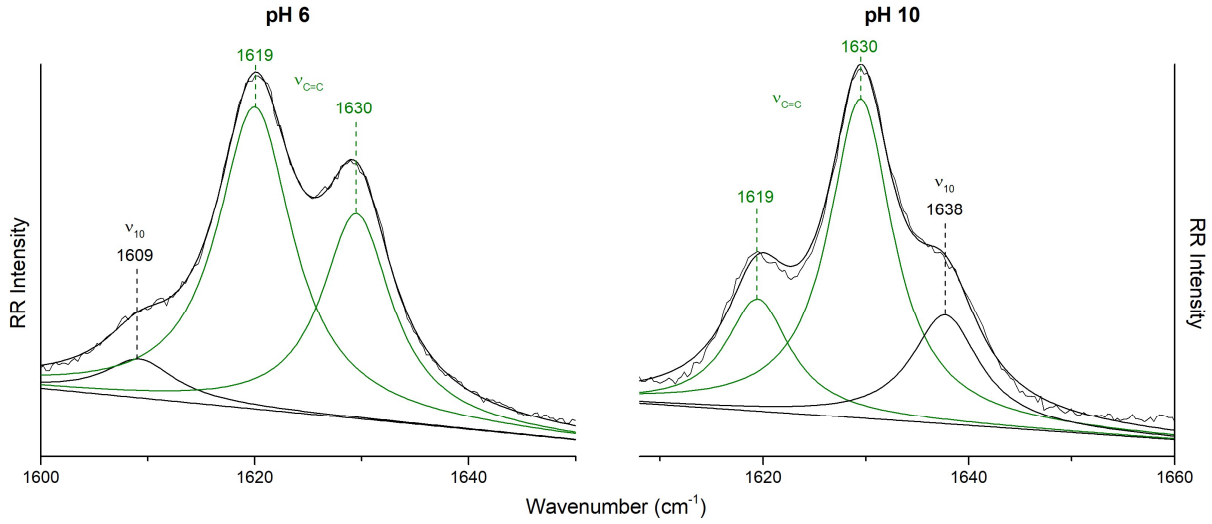

Figure S4. Spectral deconvolution of RR spectra of the ferric hNgb-H64A variant obtained at different pH values in the high wavenumber region. The vinyl stretching vibrations are reported in green. The bands are assigned according to Refs. [1-3]. The corresponding bandwidths are about 9 cm^{-1} for the vinyl stretching modes, while are within the 8-10 cm^{-1} range for the other bands.

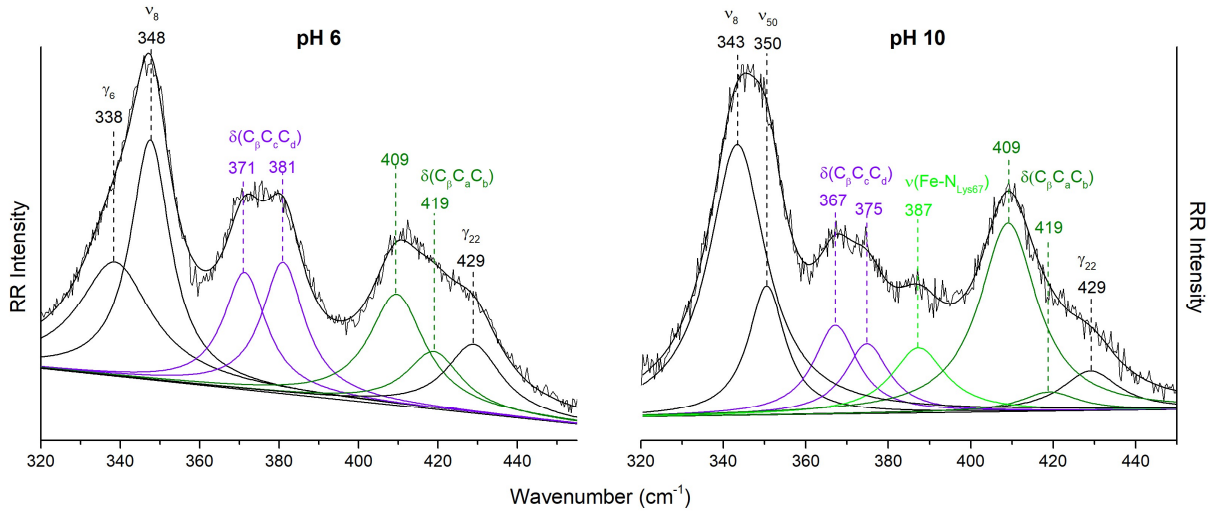

Figure S5. Spectral deconvolution of RR spectra of the ferric hNgb-H64A variant obtained at different pH values in the low wavenumber region. The propionate bending modes are reported in purple, while the vinyl bending vibrations are reported in green. The Fe-N_{Lys} stretching mode is shown in light green. The bands have been assigned according to Refs. [1-3, 5]. The corresponding bandwidths are about 13 and 17 cm^{-1} for the propionate and vinyl bending modes, respectively, while are within the 11-17 cm^{-1} range for the other bands. The only exception is represented by the γ_6 mode at 338 cm^{-1}, whose bandwidth is 22 cm^{-1}.

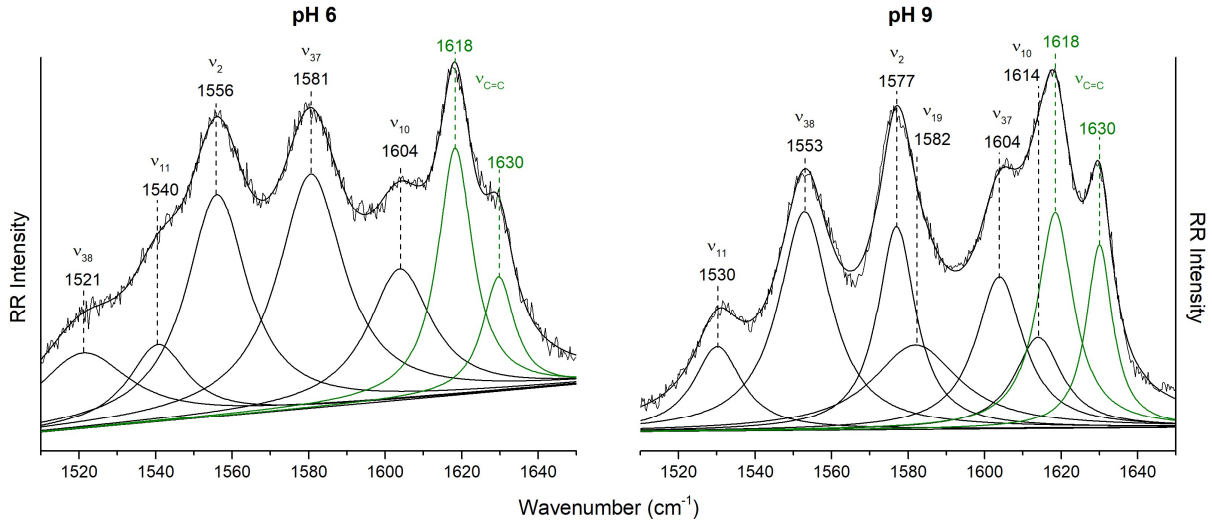

Figure S6. Spectral deconvolution of RR spectra of ferrous hNgb-H64A variant at different pH values in the high wavenumber region. The vinyl stretching vibrations are reported in green. The bands are assigned according to Refs. [1-3]. The corresponding bandwidths are about 12 cm^{-1} for the vinyl stretching modes, while are within the 15-19 cm^{-1} range for the other bands. The only exception is represented by the ν_{38} at 1521 cm^{-1}, whose bandwidth is 30 cm^{-1}.

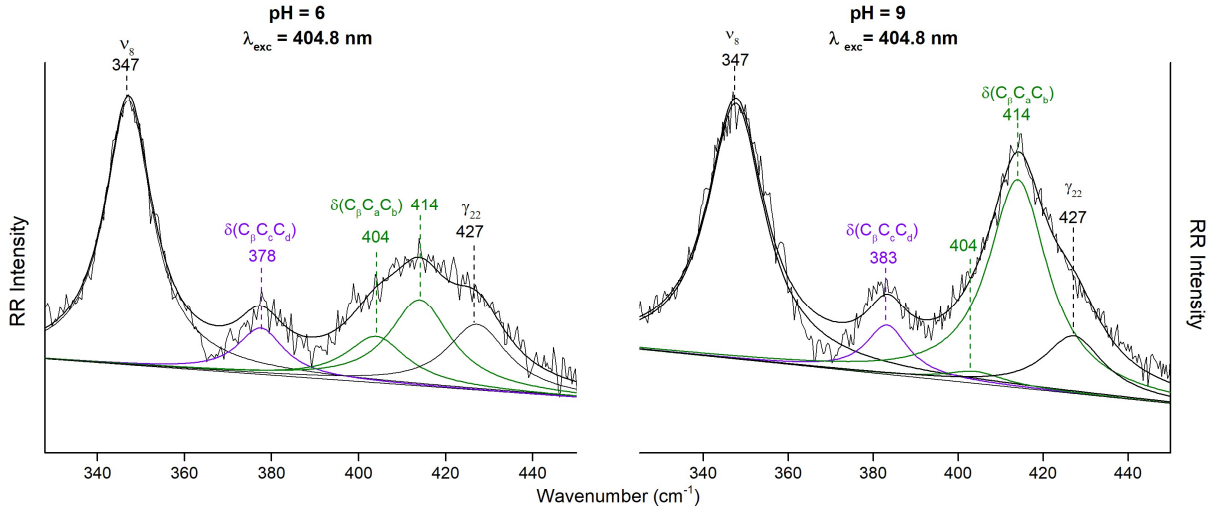

Figure S7. Spectral deconvolution of RR spectra of ferrous hNgb-H64A variant at different pH values in the low wavenumber region. The propionate and vinyl bending modes are reported in purple and green, respectively. The bands have been assigned according to Refs. [1-3]. The corresponding bandwidths are about 14 and 17 cm^{-1} for the propionate and vinyl bending modes, respectively, while are within the 11-17 cm^{-1} range for the other bands.

Table S1. Summary of the integration time (in minutes) and number of averaged RR spectra reported in this work. The spectra have been obtained with a 404.8 nm excitation wavelength, except for those marked with an asterisk, which have been obtained with a 441.6 nm excitation wavelength.

Protein	Sample	Low Wavenumber (average/integration time)	High Wavenumber (average/integration time)
WT	Fe(III), pH 12.3	10 spectra/50 min	12 spectra/60 min
	Fe(III), pH 7.4	6 spectra/30 min	6 spectra/30 min
	Fe(III), pH 3.7	10 spectra/50 min	12 spectra/60 min
	Fe(II), pH 11.4	32 spectra/160 min	6 spectra/30 min
	Fe(II), pH 7.4	26 spectra/52 min	7 spectra/14 min
	Fe(II), pH 5.2	16 spectra/80 min	4 spectra/20 min
H64A	Fe(III), pH 12.0	3 spectra/15 min	3 spectra/6 min
	Fe(III), pH 10.0	3 spectra/15 min	10 spectra/50 min
	Fe(III), pH 7.4	3 spectra/15 min	6 spectra/12 min
	Fe(III), pH 6.0	3 spectra/15 min	8 spectra/40 min
	Fe(II), pH 9.0	14 spectra/70 min	13 spectra/26 min
	Fe(II), pH 6.0	16 spectra/80 min	37 spectra/74 min
	Fe(II), pH 6.0	14 spectra/70 min*	

References

[1] S.Z. Hu, I.K. Morris, J.P. Singh, K.M. Smith, T.G. Spiro, J Am Chem Soc, vol. 115, 1993, pp. 12446-12458.
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[5] L. Milazzo, L. Tognaccini, B.D. Howes, G. Smulevich, J Raman Spectrosc, vol. 49, 2018, pp. 1041-1055.